

A progeria mutation reveals functions for lamin A in nuclear assembly, architecture, and chromosome organization

Pekka Taimen^{a,1}, Katrin Pflieger^{a,1}, Takeshi Shimi^a, Dorothee Möller^b, Kfir Ben-Harush^c, Michael R. Erdos^d, Stephen A. Adam^a, Harald Herrmann^b, Ohad Medalia^c, Francis S. Collins^{d,2}, Anne E. Goldman^a, and Robert D. Goldman^{a,2}

^aDepartment of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611; ^bDivision of Molecular Genetics, German Cancer Research Center, D-69120 Heidelberg, Germany; ^cDepartment of Life Sciences, Ben Gurion University and the NIBN, Beer-Sheva 84120 Israel; and ^dGenome Technology Branch, National Human Genome Research Institute, Bethesda, MD 20892

Contributed by Francis S. Collins, October 15, 2009 (sent for review September 10, 2009)

Numerous mutations in the human A-type lamin gene (*LMNA*) cause the premature aging disease, progeria. Some of these are located in the α -helical central rod domain required for the polymerization of the nuclear lamins into higher order structures. Patient cells with a mutation in this domain, 433G>A (E145K) show severely lobulated nuclei, a separation of the A- and B-type lamins, alterations in pericentric heterochromatin, abnormally clustered centromeres, and mislocalized telomeres. The induction of lobulations and the clustering of centromeres originate during postmitotic nuclear assembly in daughter cells and this early G1 configuration of chromosomes is retained throughout interphase. In vitro analyses of E145K-lamin A show severe defects in the assembly of protofilaments into higher order lamin structures. The results show that this central rod domain mutation affects nuclear architecture in a fashion distinctly different from the changes found in the most common form of progeria caused by the expression of LA Δ 50/progerin. The study also emphasizes the importance of lamins in nuclear assembly and chromatin organization.

centromere | chromatin | Rab1 | senescence | telomere

The lamins are type V intermediate filament (IF) proteins comprising the major structural components of the nuclear lamina, the fibrous meshwork underlying the inner nuclear membrane (1). They are major determinants of nuclear size and shape and are involved in essential functions such as DNA replication and transcription (1). Lamins A (LA) and C (LC) are alternatively spliced products of the *LMNA* gene, whereas lamins LB1 and LB2 are encoded by *LMNB1* and *LMNB2*. Structurally, the lamins have a conserved central α -helical rod domain flanked by globular head and tail domains. The central rod is responsible for the formation of in-parallel and in-register coiled-coil dimers, the building blocks of lamin polymers. In vitro, lamin dimers form head-to-tail chains, which further interact laterally in an anti-parallel orientation to form highly ordered paracrystals (PCs) (2). However, little is known about the assembly of lamins into higher order structures in vivo.

There are >250 mutations in human *LMNA* causing a wide range of diseases [for detail, see <http://www.umd.be/LMNA/> and (3)]. Among these is the rare premature aging disease, Hutchinson–Gilford progeria syndrome (HGPS). HGPS children typically appear normal at birth, but show growth retardation before the age of 2 years. Further manifestations include loss of hair, lipodystrophy, sclerodermatous skin, osteolysis, and progressive atherosclerosis leading to death at an average age of 13 years due to myocardial infarcts and strokes (4). Most HGPS patients carry the 1824C>T mutation (G608G), which activates a cryptic splice site resulting in the expression of LA with 50 amino acids deleted near its C terminus (LA Δ 50/progerin) (5). As a result, LA Δ 50/progerin remains permanently farnesylated (6, 7), and its accumulation in patients' cells is correlated with the loss of heterochromatin and changes in histone methylation (1).

In addition to 1824C>T, there are 21 other *LMNA* mutations causing progeria (<http://www.umd.be/LMNA/>). Some of these are located in the central rod domain, where they could directly impact the assembly of lamins into dimers and higher order structures. Here, we have studied the alterations in nuclear architecture and chromatin organization in one of these mutations, 433G>A (E145K), located in segment 1B of the central rod domain of LA/C (5). A patient bearing this mutation showed earlier onset cardiovascular defects, only partial loss of hair and ample s.c. fat (5). We show that fibroblasts derived from an E145K patient have severely misshapen nuclei and multiple defects in chromatin organization as reflected by centromere clustering and an abnormal distribution of telomeres throughout the cell cycle. These abnormalities are established during cell division as nuclei assemble in daughter cells. The results demonstrate that the nuclear changes in E145K progeria cells are significantly different from those seen in cells expressing LA Δ 50/progerin, and they also emphasize the essential role of lamins in establishing and maintaining nuclear architecture.

Results

Dermal fibroblasts from an E145K progeria patient (E145K cells) contain severely lobulated nuclei (Fig. 1A and Fig. S1A). Approximately 32% ($n = 300$) of the nuclei showed three or more lobules (multilobulated) by passage (p) 9, and by p13 this increased to approximately 69% ($n = 300$) (Fig. S1B). Normal fibroblasts (control cells) were essentially free of lobules [$\approx 1\%$ ($n = 300$), p15] (Fig. S1B). Contour ratio analyses confirmed that E145K nuclei had very different shapes compared to controls (Fig. S1C). Furthermore, the lobulated nuclei in E145K cells did not regain normal nuclear shapes following 48–72 h of treatment with the farnesyl transferase inhibitor FTI-277 (Fig. S2), which is distinctly different from cells expressing LA Δ 50/progerin (8, 9).

Immunofluorescence revealed that LA/C were concentrated in the lamina with lower levels in the nucleoplasm in both E145K nuclei and controls (Fig. 1A). However, in multilobulated E145K nuclei there was an overall reduction in LB1 and LB2, which were barely detectable in the outermost regions of the lamina delimiting the lobules (Fig. 1A). Any detectable B-type lamins

Author contributions: P.T., K.P., T.S., S.A.A., H.H., O.M., F.S.C., and R.D.G. designed research; P.T., K.P., T.S., D.M., K.B.-H., M.R.E., H.H., O.M., and A.E.G. performed research; M.R.E. and F.S.C. contributed new reagents/analytic tools; P.T., K.P., T.S., D.M., K.B.-H., M.R.E., S.A.A., H.H., O.M., F.S.C., A.E.G., and R.D.G. analyzed data; and P.T., K.P., T.S., S.A.A., H.H., O.M., A.E.G., and R.D.G. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

¹P.T. and K.P. contributed equally to this work.

²To whom correspondence may be addressed: E-mail: r-goldman@northwestern.edu or francis.collins@gmail.com.

This article contains supporting information online at www.pnas.org/cgi/content/full/0911895106/DCSupplemental.

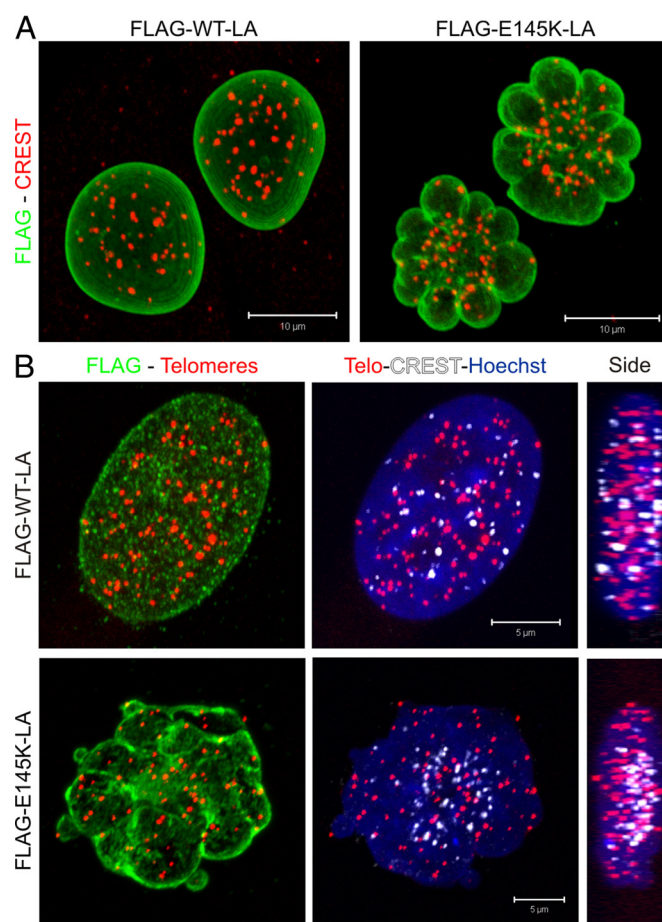
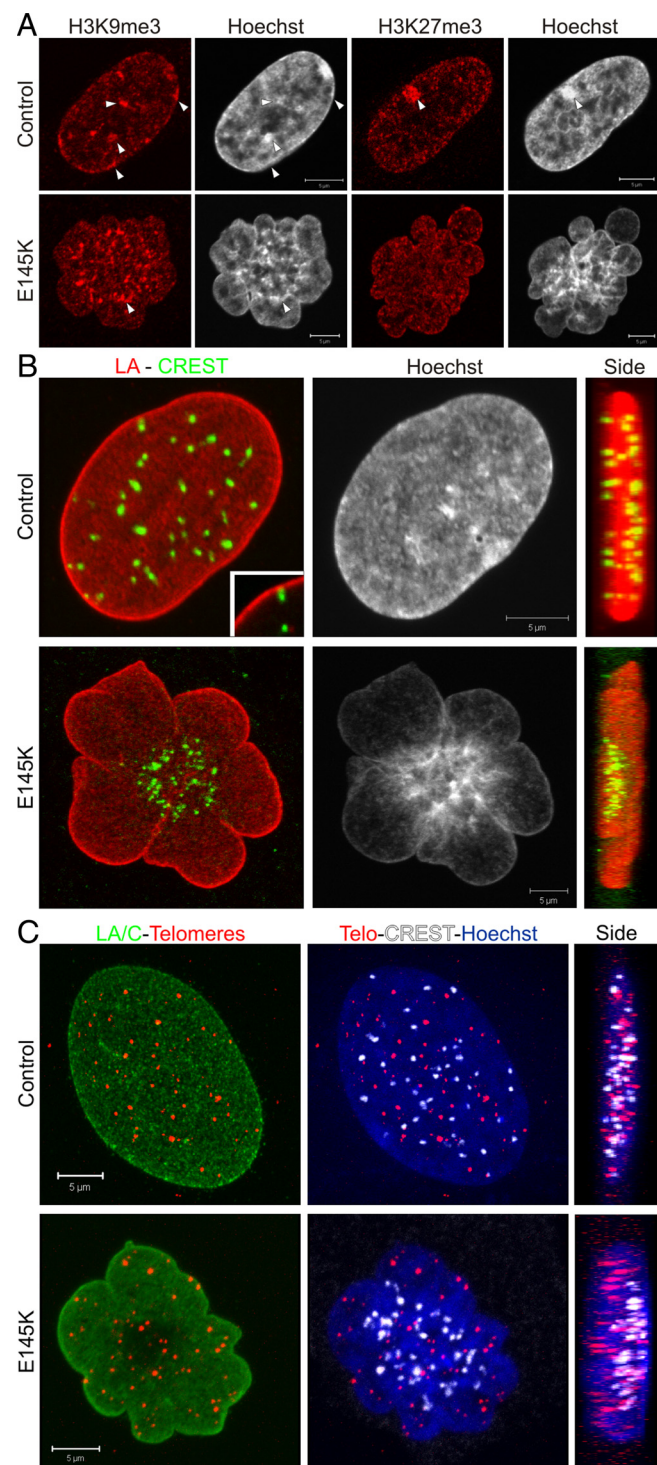


Fig. 3. Expression of FLAG-E145K-LA in HeLa cells induces nuclear lobulations and mislocalization of centromeres and telomeres. (A) HeLa cells were transfected with either FLAG-WT-LA or FLAG-E145K-LA for 72 h and stained with anti-FLAG and CREST antiserum. Maximum projections of series of z-sections spanning the entire daughter cell nuclei are shown. Note the extensive lobulation of the lamina and clustering of centromeres in FLAG-E145K-LA-expressing cells. (Scale bars, 5 μ m.) (B) HeLa cells were transfected with either FLAG-WT-LA or FLAG-E145K-LA for 72 h, fixed, and stained with anti-FLAG, CREST antiserum, Hoechst, and a FISH probe to visualize telomeres. Maximum projections of z-sections and side projections are shown as above. Centromeres and telomeres are distributed throughout the nuclei in FLAG-WT-LA-expressing cells. In FLAG-E145K-LA-expressing cells centromeres and telomeres are less interspersed, and telomeres are more likely to be associated with the peripheral lamina region. (Scale bars, 5 μ m.)

confocal section. (Scale bars, $5\ \mu\text{m}$.) (C) E145K and control cells were stained with anti-LA/C, CREST antiserum, and Hoechst. A FISH probe was used to visualize telomeres. Maximum projections of series of z-sections spanning the entire nucleus and side projections from merged images are shown. Centromeres and telomeres are distributed throughout the nuclei in control cells. In E145K cells centromeres are clustered in the middle of one region of the nucleus, while telomeres are more frequently associated with the peripheral lamina region. (Scale bars, $5\ \mu\text{m}$.)

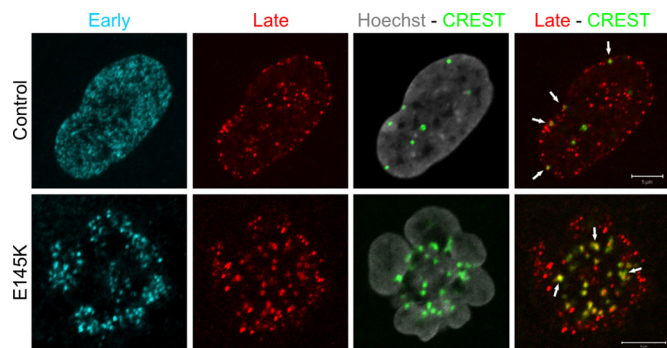


Fig. 4. Multilobulated E145K patient cells are capable of replicating their DNA despite the abnormal chromatin configuration. Double replication labeling assays (first pulse: IdU, 4 h Chase, second pulse: CldU) were carried out on E145K and control cells and subsequently stained with CREST antiserum and Hoechst. Single confocal sections showing both early and mid-to-late replication patterns are presented. In control cells, early replication foci are distributed throughout the nucleus, while mid-to-late replication foci are either found in association with the lamina region or deeper in the nucleoplasm. Some of the mid-to-late foci colocalize with CREST (arrows). In E145K cells early replication foci are enriched in lobules while many of the mid-to-late replication foci are concentrated in the central region of the nucleus with the clustered centromeres (arrows). (Scale bars, 5 μ m.)

daughter cells with multilobulated nuclei and clustered centromeres (Fig. 5 and [Movie S1](#)). Centromere clustering was maintained for the entire period of observation up to 10 h following cytokinesis (Fig. 5). In contrast, newly divided cells expressing FLAG-WT-LA always formed normally shaped nuclei with a normal distribution of centromeres within 1–2 h into G1 ($n = 12$) (Fig. S5 and [Movie S2](#)). The multilobulated nuclei induced by FLAG-E145K-LA never reverted to normal shapes following mitosis. In fact, some cells with lobulated nuclei divided to form daughter cells with lobulated nuclei and clustered centromeres (Fig. S6A). This observation was supported by flow cytometric DNA content analysis, which showed no differences in the distribution of cells in different phases of the cell cycle between FLAG-WT-LA and FLAG-E145K-LA expressing cells at 72 h

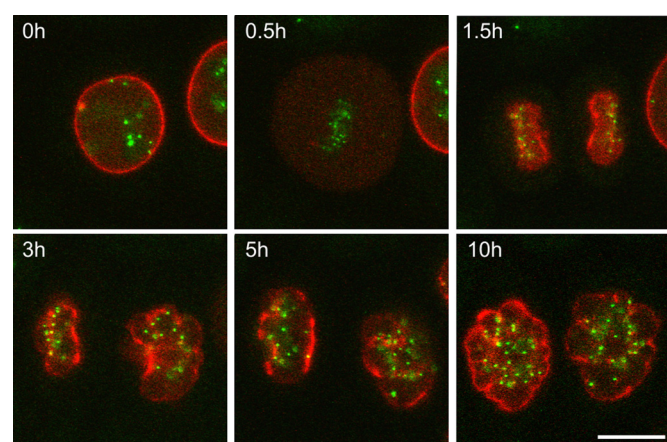


Fig. 5. FLAG-E145K-LA interferes with postmitotic nuclear assembly and centromere positioning in daughter cells. HeLa cells stably expressing YFP-CENP A (green) were transfected with FLAG-E145K-LA and mCherry-WT-LA (red) for 48–72 h. Cells with normally shaped nuclei were followed through mitosis and nuclear reassembly by using time-lapse imaging. A single confocal section is shown for each time point. mCherry-LA is localized normally during metaphase, anaphase, and telophase (0.5 to 1.5 h), but the daughter cells eventually show lobulated NE and clustered centromeres for up to 10 h. (Scale bar, 10 μ m.)

p.t. (Fig. S7). Further confirmation that the nuclear shape changes induced by FLAG-E145K-LA require cell division was obtained by arresting cells in early S phase with aphidicolin (18). This treatment completely inhibited the formation of multilobulated nuclei (Fig. S6B).

The location of the E145K mutation in the central rod domain of LA/C suggested that a disruption of normal lamin assembly might be responsible for the changes in nuclear architecture. In vitro analyses of LA complex formation by analytical ultracentrifugation (AUC) (19, 20) showed that both E145K-LA and LA Δ 50/progerin form dimers that are indistinguishable from WT-LA (Fig. S8A). To initiate assembly of higher order structures, solutions of dimers were diluted into assembly buffer, and samples were prepared at different times for TEM analysis. Both WT-LA and LA Δ 50/progerin formed extended arrays of filaments of varying diameter after 10 min (Fig. 6). In contrast, E145K-LA formed interconnected fibrous strands, which aggregated into globular disorganized assemblies. The structures formed by WT-LA and LA Δ 50/progerin at 10 min were transient states leading to larger and more ordered arrays after 30 min (Fig. S8B). These structures exhibited a characteristic 24-nm axial repeat reflecting the lateral anti-parallel association of head-to-tail chains of dimers. In contrast, some thin E145K-LA filaments appeared after 30 min, but no axial repeats could be detected (Fig. S8B).

The end point of the in vitro assembly of lamins is the formation of PCs. Due to the thickness of PCs, we determined their structure in unfixed preparations embedded in amorphous ice by using cryo-electron tomography (cryo-ET) and 3-D image processing (21). Slices (x-y) of 60-nm-thick sections through reconstructed volumes of typical WT-LA and LA Δ 50/progerin PCs showed regular arrays of protofilaments exhibiting the expected 24-nm repeats of dimeric tail domains along the ordered structures (Fig. 6). In contrast, E145K-LA formed atypical PCs with no obvious longitudinal repeat patterns demonstrating that the lateral association of protofilaments was abnormal (Fig. 6).

Since previous studies showed that LA Δ 50/progerin is less soluble than WT-LA (22), the solubility of E145K-LA was also tested. Sequential extraction of FLAG-E145K-LA and FLAG-WT-LA expressing HeLa cells, however, showed no differences in the solubilities of WT-LA and E145K-LA, suggesting that both assembled into stable structures under these conditions (Fig. S9).

Discussion

The E145K progeria mutation in LA/C alters lamin structure and assembly, inducing profound changes in nuclear architecture, a reduction in B-type lamin expression, and premature senescence. In contrast to the more common LA Δ 50/progerin mutation, the E145K mutation does not alter the posttranslational processing of the C terminus, which explains why its impact on nuclear shape is not reversed by FTI treatment (6, 7). The E145K mutation also causes the clustering of centromeres and their associated H3K9me₃, but it does not alter the distribution of H3K27me₃ heterochromatic mark. In contrast, LA Δ 50/progerin alters both of these epigenetic marks, and the association between H3K9me₃ and some centromeres is lost (12). In addition, the E145K mutation has no obvious impact on the distribution of NPCs, which is in contrast to the abnormal distribution of NPCs in LA Δ 50/progerin expressing cells (10). Therefore, distinctly different cellular phenotypes are induced by these two mutations causing premature aging.

The nuclear phenotypes induced by E145K-LA and LA Δ 50/progerin suggest that these mutations may cause progeria by altering the structure of the lamina, albeit by different mechanisms. In support of this, the E145K mutation has a deleterious effect on LA polymerization into higher order structures in vitro;

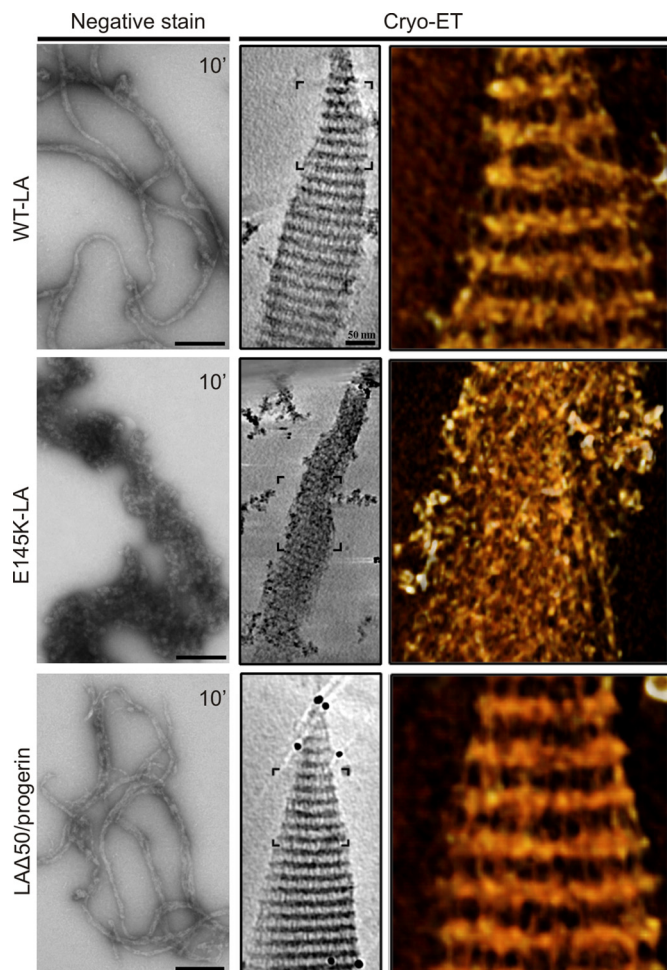


Fig. 6. Structural analysis of filaments and PCs assembled from WT-LA, E145K-LA, and LA Δ 50/progerin. Bacterially expressed and purified WT-LA, E145K-LA, and LA Δ 50/progerin were allowed to self-assemble into filaments and PCs in vitro and analyzed by TEM. Negatively stained images show that filaments of varying diameters assembled from WT-LA and LA Δ 50/progerin are well ordered after 10 min, while those assembled from E145K-LA are disorganized. For cryo-ET, 60-nm-thick x-y slices along the z-axis through tomograms of PC assembled from WT-LA, E145K-LA, and LA Δ 50/progerin are shown. Pseudo-colored higher magnification images show volume rendering views of 30-nm-thick tomographic slices of the marked regions. Both WT-LA and LA Δ 50/progerin PCs show similar repeat patterns at low magnification. In contrast the E145K-LA PC is significantly less well ordered. This is emphasized at higher magnification, which shows that the protofilaments in PCs assembled from WT-LA and LA Δ 50/progerin are well ordered, while the lateral interactions of protofilaments in PC assembled from E145K-LA are disordered. [Scale bars, 0.2 μ m (Left) and 50 nm (Middle).]

whereas the polymerization of LA Δ 50/progerin, whose amino acid sequence is significantly different from both WT-LA and E145K-LA, appears normal. Thus, it is likely that it is the abnormal association and accumulation of permanently farnesylated LA Δ 50/progerin at the NE membrane that disrupts the normal functions of the lamina in the LA Δ 50/progerin patients (6–10, 12), whereas in the case of E145K patients, it is the abnormal polymerization of LA that prevents the assembly of a normal lamina structure. Ultimately such differences could help to explain the different phenotypes seen in progeria, as well as other laminopathy patients bearing different mutations in *LMNA*.

The impact of alterations in the E145 residue are most likely attributable to its location in subdomain 1B of the central rod domain of LA/C. Similarly, mutations in the central rod domain

of cytoskeletal IF proteins, such as desmin, keratins, and vimentin, have severe consequences for filament structure and function and cause variety of diseases including skeletal and cardiac myopathies (23), skin blistering diseases (24), and cataracts (25). The E145 residue is in an ϵ position of one of the six heptads within the 42-amino acid sequence that is unique to lamins, and it is predicted to be solvent exposed rather than residing in the coiled-coil interface. Although substituting lysine for glutamic acid at residue 145 does not alter dimer assembly as shown by AUC analysis, it does alter the normal alignment of protofilaments into filaments and PCs (2). In further support of this, a mutation in the corresponding site of *Caenorhabditis elegans* lamin (*lmn-1*), Q159K, alters the structure of in vitro assembled filaments (21, 26).

As we previously observed in LA Δ 50/progerin expressing patient fibroblasts (10), nuclear shape defects accumulate as a function of passage number in E145K cells suggesting a dominant negative effect of this mutant LA/C protein. In addition, live imaging of HeLa cells expressing FLAG-E145K-LA and treatment with aphidicolin demonstrate that lobulation is established during postmitotic nuclear assembly. Therefore, it is likely that the assembly of the lamina during late telophase/early G1 is severely affected once the relative amount of the E145K mutant lamin reaches a critical level. It is also relevant to note that the E145K mutation is present in both LA and LC, whereas the 1824C>T (LA Δ 50/progerin) mutation does not affect the sequence of LC.

Within interphase nuclei, chromosomes are organized into “territories,” with gene-rich chromosomes more likely to be internally located and gene-poor chromosomes more likely to be at the nuclear periphery (27, 28). Individual chromosomes are typically oriented with their centromeres situated in the nuclear periphery (29), while telomeres are distributed throughout the nucleoplasm (30). In the multilobulated E145K, nuclei chromosomes are organized with their centromeres clustered in one region. This is reminiscent of the “Rabl configuration,” a polarized chromosome configuration found in the interphase nuclei of *Drosophila* and certain plant cells (31). The Rabl configuration originates in anaphase, when sister centromeres separate as chromosomes move to the spindle poles, and it is retained throughout interphase until the next mitosis (31). The polarized configuration of chromosomes in E145K cells is also established during and immediately following mitosis and persists throughout the G1 and S phases of the cell cycle (Fig. S10). Therefore, the expression of LA/C with the E145K mutation inhibits the normal reorganization of interphase chromosome territories in daughter cells in early G1, most likely due to the formation of an abnormal lamina structure. In support of this, it has been shown that lamins interact with mitotic chromosomes (32–34) and that their assembly at the surface of chromosomes is crucial for postmitotic nuclear formation (35, 36). It is also possible that the E145K mutation interferes with the lamin binding to matrix attachment regions of DNA (37) or interactions with other chromatin associated lamin-binding proteins such as histones, LAP2 α , BAF (33), and/or SUN2 (38). Even though we do not know the exact mechanisms responsible for the impact of the E145K mutation, the results of this study provide important insights into the role of lamins in the spatial and temporal organization of chromosomes throughout the cell cycle.

In summary, the E145K mutation causes changes in lamin polymerization and assembly, resulting in a cellular phenotype that is distinctly different from LA Δ 50/progerin expressing cells. Common features shared by these two very different mutations are that they both result in the down-regulation of B-type lamins (39) and premature replicative senescence (40). These factors may represent common denominators in the etiology of the different forms of premature aging disease. Interestingly, the expression of LA Δ 50/progerin in human adult mesenchymal

